SIGNIFICANCE

Physical methods are poised to transform drug discovery and chemical biology via quantitative, predictive design of small molecules.

Unfortunately, these methods still have severe limitations and much work is needed to expand their domain of applicability, but retrospective tests are unsuitable for this.

To truly advance these methods, we need a series of blind challenges focused on pushing the limits of predictive techniques.

SAMPL has been vital to fulfill this role; here we propose to continue and extend SAMPL via collection of a set of carefully selected experimental data in order to drive further improvements in modeling.

This work is necessary in order to advance modeling to the point where it can reliably guide drug discovery efforts, reducing time consuming and costly trial and error.

INNOVATION

We will discuss how SAMPL has previously driven innovation in the field.

Innovation here will include developing new, high-throughput experiments for studying protein ligand binding (Aim 3).

In Aim 4, we will not only run SAMPL community challenges, but also perform our own reference calculations with the latest techniques, testing their accuracy and using these to assess the current state-of-the-art.

Innovation here will also involve careful assessment of how to compare methods and analyze their relative performance in a statistically sound way.

APPROACH

Aim 1: Generate new data for "simple" SAMPL blind challenges on physical property prediction. We will develop new solution-phase datasets for druglike small molecules. These data can test critical aspects of small molecule modeling (including accounting for interactions and treatment of protonation/tautomeric state) and improve our ability to predict physical properties relevant to drug discovery in new regions of chemical space. We will initially focus on distribution between organic phases and on pKa?s and their modulation by solvent environment, using these data to drive improvements in the modeling of ligand interactions.

Aim 2: Measure binding of novel host-guest complexes for introductory ligand binding challenges. We will measure new host-guest binding free energies for cucurbiturils and deep-cavity cavitands, yielding further host-guest binding challenges which span between physical property prediction and protein-ligand binding. Host guest systems are some of the simplest cases of molecular recognition, and thus these binding data will drive improvements in modeling of simple binding systems with techniques of relevance to drug discovery.

Cucubituril-based receptors as model binding systems

Cucubituril derivatives for host-guest binding. The Isaacs group has previously participated in the SAMPL challenges and supplied unpublished host-guest binding constants [**?**, 1, 2]. Our participation was quite stimulating for us and influenced our investigation of the biomedical applications of acyclic CB[n]-type receptors (a.k.a Calabadions). Cucurbit[n]uril receptors are particularly well suited for the SAMPL challenges because they exhibit: 1) high binding constants toward suitable guests in water (routinely μM to nM; occasionally pM to fM) [3–9], 2) high selectivities between structurally related guests which translate into large $\Delta\Delta G$ values [10], 3) low molecular weights (1000-2000 amu) which allows high levels of theory to be used, and 4) limited conformational degrees of freedom. Herein, we propose to continue to participate in the next three SAMPL challenges during the proposed five year funding period by resynthesizing previously published CB[n]-type receptors of increasing complexity, measure Ka values and determine host-guest stoichiometry and geometry toward biologically relevant guests which will allow the computational chemists to push the boundaries of the free-energy prediction of receptor?ligand complexes. Figure 1 shows the chemical structures of three hosts – Me4CB[8] [11], glycoluril hexamer [12], and acyclic CB[n]-type receptors [13–18] which span the range from preorganized macrocyclic host to uncharged acyclic but preorganized host to highly charged acyclic host.

SAMPL6. For this challenge we propose to measure K_a and ΔH values, stoichiometry, and geometry for the interaction of Me4CB[8] (nicely water soluble CB[8] derivative) toward 15 guests (chosen from top selling drugs, Table CB1) by either direct or competition isothermal titration calorimetry (ITC), UV/Vis or fluorescence indicator displacement assay, or NMR competition experiments which we are very experienced with [3, 4, 19, 20]. Our selection of Me_4CB[8] and top 100 drugs was based on a desire to increase the level of complexity of the

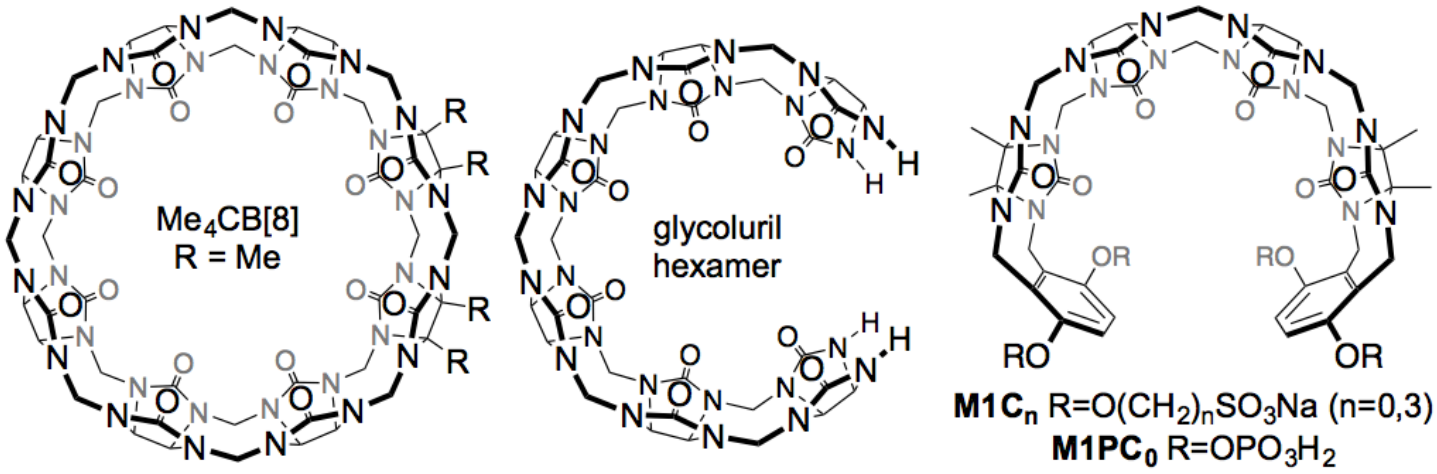

Figure 1: **Structures of Me_4CB[8], glycouril hexamer, and acyclic CB[n]-type receptors.**

computational challenge by: 1) changing host flexibility (e.g. Me_4CB[8] can exhibit ellipsoidal deformation) [11], 2) by allowing the possibility of binary or ternary (e.g. 1:1 and/or 1:2 host:guest) complexes [21–23], 3) using drugs with several potential binding epitopes to include sampling issues. Host:guest stoichiometry and geometry (e.g. which binding epitope is complexed) will be addressed by ITC "n" values, Job plots monitored by UV/Vis or NMR [24], and by 1H NMR complexation induced changes in chemical shifts [25]. All three sets of studies will be conducted in phosphate buffered saline (pH 7.4 with physiological salt) which introduces further complexity due to competitive interaction between the C=O portals of CB[n]-type receptors and metal ions via ion-dipole interactions which reduces the observed Ka values [26].

drug	features
memantine	adamantane; 1:1
saxagliptin	adamantane; 1:1
premarin	steroid
pancuronium	steroid
varenicline	1:1 vs 1:2
valsartan	pKa 4.37
omeprazole	pKa 4.77
ranolazine	pKa 7.17; epitopes
pradaxa	pKa 3.87; epitopes
nilotinib	epitopes; pKa 6.3
sensipar	epitopes; folding
vyvance	diamine; epitopes; folding
minocycline	tetracyclin; amino aniline

Table 1: Selected drugs as guests

SAMPL8. We propose to study host:guest complexes of glycoluril hexamer toward the 15 drugs (Figure CB1). We select glycoluril hexamer for this challenge because it: 1) increases the conformational dynamics of the host, and 2) influences the number and energy of solvating (and unusually coordinated) water molecules that are implicated in the observed high binding constants for CB[n]-guest complexes [9, 27]. Furthermore, in selecting the drugs, we have chosen several that have pKa values in the 3.8 to 7.4 range. Similar to biomolecular host-guest systems, CB[n]-type receptors are well known to induce pKa shifts (up to 4 pKa units) of complexed guests [28–30], and the ability of computation to replicate and predict such shifts and their impact on Ka are of high significance.

SAMPL10 We will focus on acyclic CB[n]-type receptors (e.g. $M1C_3$, $M1C_0$, and $M1PC_0$ that contain anionic solubilizing groups attached via different linker lengths. As in SAMPL2, these acyclic CB[n]-type receptor introduces conformational complexity and influences the free energy of the solvating H_2O molecules in the free host. Moreover, the presence of 4 anionic groups in close proximity to the cavity are expected to have a significant influence on the balance between ion-dipole interactions and the solvation of the free host.

Gibb deep cavity cavitands for host-guest studies

History of octa-acid SAMPL challenges. During SAMPL4 [31] and SAMPL5 [32] we focused on two hosts: the octa-acid 1 (R = H) and another octa-acid derivative with four methyl groups positioned at the portal of the binding pocket (1, R = Me). These studies used Isothermal Titration Calorimetry (ITC) to measure the thermodynamics of (1) host 1 (R = H) complexing a range of carboxylate guests, and (2) the binding of carboxylate and trimethylammonium guests to both hosts (1, H = H and Me). In both cases ^{1}H-NMR titration was also used in a confirmatory role for ITC-derived free energies of binding. SAMPL5 emphasized how differences in the shape of the hydrophobic pocket of the host can have a profound affect on affinity for some guests.

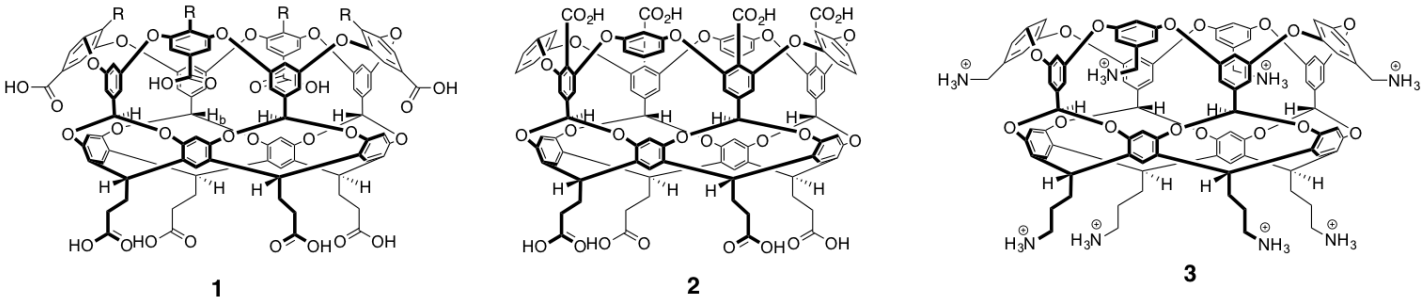

Figure 2: **Gibb deep cavity cavitands for SAMPL6-10.**

Novel deep cavity hosts probe the effects of binding site charge constellations. For future SAMPL challenges, we will expand on the range of hosts by including 2 and 3 in our ITC studies. Like cavitand 1, host 2 is an octa-acid derivative. However, the four benzoate groups are relocated from the extreme exterior in the case of 1, to the rim of the binding pocket in 2. We surmise that this will have a direct effect on the binding of charged guests, but more subtly, an indirect effect on guest complexation via changes to the solvation of the empty host. Octa-trimethylammonuim cavitand ("positand" 3) has the same overall architecture as host 1, but inverts the charges on the water solubilizing exterior coat. While it is not yet clear if this switch in groups relatively remote from the pocket will directly affect guest complexation, results from related systems suggest it can (unpublished).

Guests for the five proposed ITC studies will be obtained from commercial sources, focusing on molecules that probe the limitations of current force-fields as well as new data as it is gathered.

SAMPL6-10 deep cavity cavitand challenges. The host-guest challenge for SAMPL6 will focus on how well the effect of host carboxylate substituent location can be predicted, and will involve hosts 1 and 2 with a set of five, previously uninvestigated guests. SAMPL7 will provide a second iteration of this experiment to test algorithmic improvements in predictive modeling following SAMPL6 by comparing hosts 1 and 3 with a different set of guests. We anticipate that because of the relative remoteness of the charged groups in these two hosts, the effects of switching charges will be subtler than the differences between 1 and 2. SAMPL8 will consider the effect of common biologically-relevant counterions/salts salts on guest binding, comparing the effects of NaCl and NaI on the complexation of five guests to 1. We have previously shown that iodide has a weak affinity for the binding pocket of 1, whilst sodium ions have an affinity for the outer carboxylates [33], requiring modeling to capture the differential affinities of these ions in addition to guest affinities to successfully model the observed affinities. SAMPL9 will follow up on this by examining the effects of these same two salts on the complexation of five guests to 3, again giving the modeling community time to incorporate algorithmic improvements following SAMPL8. While we have not yet quantified salt affinities to host 3, we expect the iodide to have affinity for both the pocket and the positively charged solubilizing groups. For SAMPL10 we will consider the effects of co-solvents on the binding of five guests to 1 and 2 to probe the effect of co-solvent competition for the binding site, as well as effects cosolvents may have in weakening the hydrophobic effect.

Aim 3. Generate biologically relevant advanced model systems for protein-ligand binding challenges. We will identify suitable biological protein-ligand model systems (difficult but tractable in order to push the limits of physical techniques) then measure binding and develop these for blind challenges. This will include binding studies on human serum albumin and bromodomains or aspartyl proteases; initial binding data will be expanded by the selection of additional ligands or the creation of mutations in the protein that modulate binding.

Aim 4. Coordinate, run, and analyze blind challenges to advance modeling of binding. The data collected in Aims 1-3 will drive annual SAMPL blind challenges, allowing the field to test the latest methods and force fields to assess progress, compare them against one another head-to-head, and perform sensitivity analysis to learn how much different factors (protonation state, tautomer selection, solvent model, force field, sampling method, etc.) affect predictive power. Results will then feed back into improved treatment of these factors for subsequent challenges, driving regular cycles of application, learning, and advancement.

TIMELINE

COLLABORATION MANAGEMENT PLAN

OUTLOOK

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